Response Form for the Final Performance Review Report*

1. Name of Grantee: Fox Chase Cancer Center

2. Year of Grant: 2008 Formula Grant

A. For the <u>overall grant</u>, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an "unfavorable" rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

Upon grant award, all report deliverables are noted in database format. Three months prior to agency deadlines, an electronic memo is sent to the individual responsible for providing the documentation. Concurrently, a Grants Specialist is assigned to assist the Principal Investigator with formulating the response and applicable regulation and compliance issues. A single individual is responsible for collating and submitting all progress and final reports to the Bureau of Health Statistics and Research at the Pennsylvania Department of Health.

Oversight of the research progress is conducted via Working Group reviews of scientific programs. Each investigator is part of at least one Working Group. These groups have bimonthly meetings at which they present ongoing projects to a group of their peers who share similar research interests. The meetings serve as a forum for critiquing work, gaining additional scientific perspective, and solving research issues that could otherwise present obstacles to successful execution of the proposed program. Given this ongoing oversight, if a Principal Investigator receives an "unfavorable" rating and cannot provide evidence of exceptional circumstance that prevented the proposed work from being carried to fruition, they are disqualified as future recipients of CURE funding.

* Please note that for grants ending on or after July 1, 2007, grantees' Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

Project Number: 0863401
Project Title: A Growth-Regulating Protein Tyrosine Phosphatase
Investigator: Chernoff, Jonathan

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

<u>Reviewer 1</u>:
In general, the goals attained were consistent with the available resources provided.

<u>Reviewer 2</u>:
None

<u>Reviewer 3</u>:
None

<u>Response:</u>
There were not any weaknesses or recommendations listed in Section B. We thank the reviewers for their feedback.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: None

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None

Project Number: 0863402
Project Title: Characterization of the Role of MTAP Gene in Tumorgenesis
Investigator: Kruger, Warren

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

<u>Reviewer 1</u>:
The project was successful, but additional publications would have made this outstanding.

<u>Response:</u>
The major weakness identified by all three Reviewers is the lack of peer-reviewed publications. We have just published part of this work in a paper published in PlosOne entitled "Germline Mutations in Mtap Cooperate with Myc to Accelerate Tumorigenesis in Mice" (8(6):e67635, 2013). We are currently working on a new MTAP manuscript tentatively entitled "Loss of MTAP promotes tumorigenesis by a mechanism unrelated to its known enzymatic activity".

Hopefully this will get out this year. In general, my lab does not like to publish small publication units and prefers to wait until the work is mature.

<u>Reviewer 2</u>:
1. A considerable amount of funding was provided for this project. One would expect more progress, as measured by submissions and publications of peer-reviewed journal articles, for this level of funding. The one published paper apparently derived from results obtained before the research period for this grant began.

2. The researchers should address the variability in experimental results mentioned above, in terms of pinning down the source of variability, leading to results that will be convincing and publishable. Overall conclusions and future directions should be clearly spelled out at the end of the progress report.

<u>Response:</u>
With regards to Reviewer 2's comment about pinning down the source of variability, we entirely agree. What we do know is that the variability is not technical in nature, but stems from variability within the animals. Because the mice are inbred, we believe it is unlikely that it stems

from genetic differences in the animals. In general, getting a handle on stoichastic sources of biological variability is very difficult, and usually must be dealt with statistically.

Reviewer 3:
It would help if additional outside funding and peer-reviewed publications were produced.

Response:
The major weakness identified by all three Reviewers is the lack of peer-reviewed publications. We have just published part of this work in a paper published in PlosOne entitled "Germline Mutations in Mtap Cooperate with Myc to Accelerate Tumorigenesis in Mice" (8(6):e67635, 2013). We are currently working on a new MTAP manuscript tentatively entitled "Loss of MTAP promotes tumorigenesis by a mechanism unrelated to its known enzymatic activity".

Hopefully this will get out this year. In general, my lab does not like to publish small publication units and prefers to wait until the work is mature.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response:

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: We very much appreciate the support for our research by CURE.

Project Number: 0863403
Project Title: The ARF Tumor Suppressor and Autophagy
Investigator: Murphy, Maureen

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

<u>Reviewer 1</u>:
Although not a real weakness per se, there is a need to pursue the biological consequences of the use of PAS and its derivative in whole animals, including a comprehensive dose response and lethal/toxic dose study if this has not been completed. A study of the adverse effects is also essential for translation to human use.

<u>Response</u>: With regard to the comments about the importance of testing these HSP70 inhibitors in animal studies, we recently published our study demonstrating the efficacy of PAS in a mouse model of cancer, and comparing it to PAS-Cl (note: these are denoted PES and PES-Cl in the manuscript, and below). The efficacy of PES versus PES-Cl in B cell lymphoma was published this year (Balaburski GM, Leu JI, Beeharry N, Hayik S, Andrake MD, Zhang G, Herlyn M, Villanueva J, Dunbrack RL Jr, Yen T, George DL, Murphy ME. A modified HSP70 inhibitor shows broad activity as an anticancer agent. Mol Cancer Res. 2013 Mar;11(3):219-29. doi: 10.1158/1541-7786.MCR-12-0547-T. Epub 2013 Jan 9. PubMed PMID: 23303345; PubMed Central PMCID: PMC3606282.). Briefly, we reported that PES-Cl can significantly increase the lifespan of mice in this study. We also found that PES-Cl was far superior to PES. Notably, we found no evidence in these animals for any evidence of toxicity, in the liver or other organs. We recently procured funding to analyze PES and PES-Cl in mouse models of melanoma (P01 CA11404-06, Targeted Therapies in Melanoma, PI Herlyn).

<u>Reviewer 2</u>:
The primary reservation relating to this work is that the proposed animal studies relating to tumor development were not performed. Furthermore, although the papers published were of high quality, the level of productivity appears to be somewhat low considering the considerable amount of funding that has been made available through both the Pennsylvania Department of Health and the National Institutes of Health.

<u>Response</u>: With regard to the level of productivity, though it did take longer than usual, we did

recently publish our study on the identification of the ARF autophagy domain. Notably, this manuscript was published in a high quality journal (Autophagy, impact factor = 12). Importantly, we discovered that tumor-derived mutations of ARF that do not alter p16 coding region all impair the ability of ARF to induce autophagy. This is the first demonstration that exon 2 mutations in the CDKN2A gene can target ARF and autophagy, instead of p16INK4a, as previously believed (Budina-Kolomets A, Hontz RD, Pimkina J, Murphy ME. A conserved domain in exon 2 coding for the human and murine ARF tumor suppressor protein is required for autophagy induction. Autophagy. 2013 Aug 7;9(10). [Epub ahead of print] PubMed PMID: 23939042).

Reviewer 3:
Recommendation (not a weakness): Further development of the drugs is highly warranted, including pharmacokinetic and pharmacodynamic (PK/PD) studies and the possible introduction of the compound in clinical trials.

Response: We appreciate this Reviewer's enthusiasm for this study and we fully plan to conduct PK/PD studies on PES and PES-Cl.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response:

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: I greatly appreciate the comments of the Reviewers. We think our HSP70 inhibitor is a truly exciting drug, and are eager to see it one day in clinical trials for cancer. I remain grateful for the support from the Pennsylvania Department of Health.

Project Number: 0863404
Project Title: Anti-Glucose Transporter-1 Antibodies as a Novel Treatment against Human Cancers
Investigator: Simon, George

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

<u>Reviewer 1:</u>

1. The investigators may like to refer to the following paper that outlines potential strategies for use of glycolysis as target for therapy (Gatenby RA, Gillies RJ. Glycolysis in Cancer: A Potential Target for Therapy. *The International Journal of Biochemistry & Cell Biology* 2007; 39:1358-1366).

2. The investigator should state if this line of work will be continued and if not what may be the major scientific obstacles that were identified through his initial attempt. The investigator should state how another investigator can continue this work or whether it should be continued at all by any other investigators.

3. The project ended prematurely. There were no publications and no additional funding. The aims were only partially achieved, but the exact details are missing. Apparently, the proposed line of work will not be continued.

<u>Reviewer 2:</u>

1. The most serious concern is that there is little evidence that the work was conducted as proposed. In fact, the application and progress reports provide substantial evidence that the converse may be true. It is therefore difficult to provide specific recommendations for improvement without more specific detail. At a minimum, the work should be completed as proposed and fully presented.
2. In addition to the under productivity or nonproductivity issues above, the experimental design was largely descriptive and incompletely developed. Specific weaknesses included lack of proper controls and corresponding metabolic studies. These should be individually addressed. The feasibility and efficacy of systemic disruption of GLUT1 function have also not been established. The most direct approach might involve the generation and testing of

conditional GLUT1-deficient adult mice. If successful, this would validate the viability and potential efficacy of this approach and would remove a number of ambiguities associated with unsuccessful GLUT1-directed antibody or small molecule attempts.

3. There was no demonstrable research productivity that can be directly attributed to this work. No abstracts, publications, or grant applications are listed. The project failed to meet any of its stated objectives, and there is little, if any, evidence that acceptable progress was made in meeting them.
4. Tangible benefits arising from this project are highly unlikely for reasons outlined above.

Reviewer 3:

1. In general, the premise for this particular project was not based on strong preliminary data. The PI had one publication (Cancer Letter, 2007) that suggested the utility of targeting GLUT1 for the treatment of breast cancer. However, this publication lacked several critical controls (i.e., testing the impact of anti-GLUT1 on glucose transport at early time points), testing the impact of anti-GLUT antibodies on glucose transport and cell viability/proliferation in cell lines lacking GLUT1 (such as U937) or normal cells. And it did not utilize alternative RNAi based strategies to validate the role of GLUT1 in facilitating glucose transport and/or viability in the tested cell lines. The absence of a robust system to test target specificity and efficacy therefore undermined the proposed research strategy. This, in addition to minimal preliminary data (impact on pAMPK and pAKT), did not strengthen the rationale for Specific Aim 1. While work from other groups continues to substantiate the utility of targeting GLUT1 for cancer therapy, this project failed to take into account testing the impact of developed agents on normal cell types, and they appear to have realized that after the project was initiated. Subsequent efforts at targeting GLUT1 seem to have been directed towards identification of a tumor specific GLUT1 epitope but were terminated with the closure of the PI's laboratory.

2. While the effort towards targeting GLUT1 is worthy of investigation, the PI did not have the right systems in place to evaluate critically the target specificity of the GLUT1-directed agents or to evaluate efficacy. Critical evaluation of prior publications and preliminary data appears to be lacking prior to funding this proposal.

3. The PI has closed his research lab and moved to a new institution; therefore, there are no recommendations for future improvement of this particular project.

Response:

We appreciate the reviewers' thoughtful feedback on this project and recognize that not all of the objectives were achieved. With the approval of the PA DOH, this project was terminated early and the remaining funding was utilized to support another approved project. This was necessary due to the closure of the PIs research laboratory.

Due to the subsequent relocation of the PI to another institution, we are unable to provide a response regarding the project-specific weaknesses and recommendations outlined in the performance review.

Recommendations for Fox Chase Cancer Center

Reviewer 2:
It is not clear whether Dr. Simon's lack of productivity could be attributed, in part, to either competing professional obligations or lack of protected time or support. It is also not clear how rigorously the protocol was reviewed for scientific merit prior to initiation or whether institutional or departmental processes were in place to monitor progress and address barriers to successful completion. Institutional core resources played critical roles in both aims, but their proportionate responsibility and accountability for a lack of demonstrable research products are also not readily apparent. In other words, does Fox Chase Cancer Center share some responsibility with the PI for the lack of acceptable research productivity?

Response:
As mentioned above this project was terminated early, with the approval of the PA DOH, before all stated objectives could be achieved. This was necessary due to the closure of the PIs research laboratory so that he could dedicate his full-time effort to clinical research and patient care. The institution was careful to follow all PA DOH procedures for the termination of this project.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response:
Formula funds are allocated for research projects based on scientific merit, investigator expertise, and the potential for leveraging future external funding. The project selection team is aware of the criteria used to evaluate the projects by this mechanism. Going forward selections will continue to be based on these criteria to ensure that projects are of high quality and that the investigator is committed to its success. Furthermore, the investigator involved in this project is no longer employed at our institution and will not be included in any future grant applications.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.

Project Number: 0863405
Project Title: Regulation of Human Somatic Wee1 by Cyclin A/Cdk2 Complexes
Investigator: Enders, Greg

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Summary Report using the following format. As you prepare your response please be aware that the Final Performance Review Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

<u>Reviewer 1</u>:
It appears that the basis for the project may not have been quite as sound as proposed. It is not clear to me how compelling this problem is, going forward.

The project had no publications or grant submissions, limited scientific impact, and no community outreach.

<u>Response</u>:
The investigator has identified a novel regulatory region that is conserved in somatic Wee1 proteins of vertebrates and functionally distinguishes them for the first time from embryonic Wee1 proteins. Mutation of this region has validated a non-canonical role of Wee1 in regulating DNA replication, as suggested in recent work by Sorensen and Syljuasen and others, and suggested that Wee1 may play a key role in regulation of DNA damage responses. This work dovetails neatly with emerging evidence that Wee1 may be a useful target to improve cytotoxic chemotherapy, the backbone of cancer treatment. A key Wee1 knockin model has been generated, allowing rigorous analysis of the role of this regulatory region in embryo fibroblasts and the intact animal.

The approach used emerging Zinc finger technology to generate highly specific knockin mutations of a novel regulatory region of mouse Wee1. Prior Wee1 knockout mice have exhibited embryonic lethality. A particular strength of this new technology is its ability to efficiently generate multiple knockin mutations of a small genetic element. As with other new technologies, a need for some troubleshooting is to be expected. Sigma initially provided a Zn finger binding site that was too far from the desired mutation site to provide optimal efficiency. This has been recognized during beta site testing at Fox Chase by Dr. Dietmar Kappes. Sigma has now provided us with primers closer to the mutation site, which should solve this problem for the planned additional knockin mutations.

The Merck Wee1 Inhibitor MK-1775 has enhanced the efficacy of cytotoxic chemotherapy in early clinical trials (Hirai et al., 2009, 2010). Our colleagues, Tim Yen and Neil Beeharry, have discovered an FDA-approved drug that chemosensitized pancreatic carcinoma cells to gemcitabine chemotherapy in vitro and in mouse xenografts. Together with our colleague Jeffrey Peterson, Yen and Beeharry have discovered that this drug has unrecognized off-target inhibition of Wee1. This work is in submission. In total, eight papers published in the past 4 years from several different laboratories have implicated Wee1 as a promising drug target for cancer therapy, with our colleagues unpublished work to follow.

We successfully generated from this project a Wee1 knockin mouse line that has revealed Wee1 role in markedly modulating DNA damage responses, with strikingly opposing protective or deleterious effects to distinct agents. This goal was accomplished shortly after the end of the period of funding by this mechanism, with the generation of a Wee1 site 1 knockin mutation.

A manuscript is in preparation for submission within the next 6 months to a competitive journal. An NIH R01 grant application has been submitted, a multi-PI NIH grant application with Dr. Yen. The scientific impact of the work is summarized above. Community outreach has no relevance to this basic science project. Instead, the new NIH R01 application seeks funding to develop new strategies based on the project to enhance cytotoxic chemotherapy of cancer, an important societal need.

Reviewer 2:
Aim 1 should not rely solely on the generation of reagents such as antibodies.

The experimental design should have a "Pitfalls" section, and other techniques/experimental designs need to be implemented rapidly. Better validation using *in vitro* studies and better design of the targeted sequence could have been more successful.

For Aim 2, generating mice knock-in mutant lines can be long and tenuous.

Recommendation: Validation using either pre-clinical models or a stable expression using Cell lines could have been an alternative for studying the effect on cell proliferation.

Response:
We raised specific antibodies against a phosphorylation on Wee1. These antibodies have proven to be highly sensitive and specific. They cross-react well with both human and mouse Wee1.

The design of the targeting sequence was dictated by constraints beyond the investigator's control: the distant location of the original Zinc finger binding sites, as supplied by Sigma, and a region of the DNA that could not be cloned or even synthesized well on the other side of the mutation target region. Nonetheless, we persevered with this approach and it succeeded shortly after the end of the funding period. This issue is now moot, as Sigma, in response to our work and others, provided Zinc finger pairs close to the mutation site.

Perseverance in generating the knockin line led to the key breakthrough in the project.

Reviewer 3:
1. The structure of this grant is flawed in terms of time frame, as the PI acknowledges. Two years is not sufficient time for the generation and characterization of mouse models. However, this reviewer does not have a problem with this type of grant funding the initial establishment of mouse models.

2. There are several specific/technical problems with the proposal. It is unclear why Sub aims 1a, 1c, 1e, and 2a were not completed. Sub aim 1a requires only simple interaction experiments (co-IPs, GST pulldowns, etc.), which are not difficult. Likewise, Sub aim 1c requires only cell cycle synchronizations. Sub aim 1e requires only that the experiments used as preliminary data for Wee1 be applied to Cyclin D. The technical impasse described for Sub aim 2a should easily be overcome through the use of retrovirus-mediated gene transfer. These experiments are all simple and could have easily been completed within the two-year grant.

3. The outsourcing of certain tasks to commercial vendors is somewhat questionable in terms of expense and time saved.

Response:
1. The key mouse model was successfully established shortly after the funding period.

2. The investigator made the strategic decision to focus most on generating the mouse knockin line, because of its irreplaceable potential for rigorous and functional analysis of the impact of the regulatory region. The investigator believes that the outcomes of studies with this line have validated this judgment. Much of the remaining work on the Aims has now been accomplished, but in ways focused by the phenotype of the knockin line.

3. The investigator outsourced production of an antibody, a task with which his lab does not have expertise. A vendor was chosen with an excellent local track record. This antibody has proved to be a robust reagent, exceeding project needs. This investment continues to pay off in providing an abundant supply of a sensitive and specific reagent that allows monitoring the key phosphorylation on Wee1.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response:

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: